UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PREVENTION, PESTICIDES, AND TOXIC SUBSTANCES
WASHINGTON, D.C. 20460

November 10, 1999

MEMORANDUM

SUBJECT: **ACIFLUORFEN: TOXICOLOGY CHAPTER FOR RED**

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TO: Virginia Dobozy
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PC Code No.: 114402
DP Barcode No.: D252559
Submission No.: S555157

ACTION REQUESTED: Prepare a toxicology chapter for the Acifluorfen RED.

RESPONSE: The toxicology database for Acifluorfen has been reviewed by the Reregistration Branch 1. The database has undergone QA/QC by the Toxicology Science Advisory Council (TOX SAC) on December 8, 1998 and peer reviewed by the Health Effects Division's Hazard Identification Assessment Review Committee (HIARC) on January 19, 1999 and February 11, 1999 and by the HED FQPA Safety Factor Committee on September 11, 1999. Although there is a data gap for a developmental neurotoxicity study, the toxicology database for Acifluorfen is adequate to support a Reregistration Eligibility Decision (RED). The toxicology chapter for the Acifluorfen RED is attached.

ACIFLUORFEN: TOXICOLOGY CHAPTER FOR RED

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ACIFLUORFEN: TOXICOLOGY CHAPTER FOR RED

I. TOXICOLOGY DATA BASE

HAZARD PROFILE

The toxicological data of Acifluorfen is adequate to support reregistration eligibility. However, the HIARC recommended a developmental neurotoxicity in rats be conducted based on indication of neurotoxicity characterized by increased incidence of dilated lateral ventricles of the fetal brain in the rats of a developmental toxicity study in rats (MRID No. 00122743). In addition, no neurotoxicity studies are available for acifluorfen and for structurally related compounds (oxyfluorfen, nitrofen, fomesafen, and lactofen) which might provide an understanding on the effects of acifluorfen on the developmental nervous system.

ACIFLUORFEN (Sodium 5-[2-chloro-4-(trifluoromethyl) phenoxy]-2-nitrobenzoate) is the active ingredient of two herbicides, Tackle and Blazer, which were originally manufactured by two companies. Both Tackle and Blazer are currently registered for use on food crops.

Toxicological data are available on both products; however, the data on Tackle are more current, complete, and acceptable. Therefore, discussions of the toxicology of acifluorfen are predominantly based on the data derived from the studies with Tackle, and where appropriate the data on Blazer are also utilized. It should be noted that Tackle contains approximately 20% to 24% of acifluorfen as the active ingredient, whereas Blazer contains approximately 40% of acifluorfen.

The acute toxicity data indicated that acifluorfen had low acute oral, dermal and inhalation toxicity. It was not a skin sensitizer. However, it caused severe eye and moderate skin irritation.

The subchronic feeding study in rats and mice showed a decrease in body weight and signs of liver toxicity (characterized by increased liver weight and increased incidence of cellular hypertrophy).

The chronic feeding toxicity study in rats, mice, and dogs demonstrated that acifluorfen induced liver toxicity (acidophilic cells in the liver and increased liver weight) and kidney toxicity (nephritis/pyelonephritis and increased kidney weight). An increase in the incidence of stomach ulcer was also seen in chronic feeding study in rats.

The carcinogenicity data showed that acifluorfen produced a statistically significant increase in the incidence of liver and stomach tumors in mice but not in rats. Acifluorfen was classified as a Group B, probable human carcinogen, and the unit risk [q^*_1] was calculated to be 3.55×10^{-2} $(mg/kg/day)^{-1}$.

Acifluorfen produced developmental toxicity (decreased fetal body weight and the increase in anatomical variations) in rats but not in rabbits, and it did not affect the reproductive parameters in rats.

The acceptable genetic toxicology studies indicate that sodium acifluorfen was weakly mutagenic in *Salmonella typhimurium* TA100 at high S9-activated concentrations and weakly recombinogenic in *Saccharomyces cerevisiae* at high nonactivated concentrations but was negative for gene mutations in

Chinese hamster ovary (CHO) cells. The test material was also negative for clastogenic effects *in vivo* and did not induce unscheduled DNA synthesis in primary rat hepatocytes. Although sodium acifluorfen induced Y chromosome loss and dominant lethal mutations in *Drosophila melanogaster*, the concern for possible heritable effects is lessen by the negative results of the rat dominant lethal assay. The acceptable studies satisfy the pre-1991 mutagenicity guideline requirements. However, because the biological relevance of the positive bacterial assay data is not clear, it is recommended that sodium acifluorfen be tested in the pre-incubation modification to the *Salmonella typhimurium* mammalian microsome gene mutation assay.

Acifluorfen was rapidly absorbed orally and eliminated mainly in the urine (46-58% of the dose) and feces (21-41% of the dose). The major component present in urine and feces was unchanged acifluorfen and amine metabolite, respectively. No tissue accumulation was observed.

The toxicology database provides sufficient information for selecting various toxicity endpoints and doses for assessing the risks for this chemical (page 23). The Health Effects Division (HED) FQPA Safety Factor Committee met on September 13, 1999 to evaluate the hazard and exposure data for acifluorfen and recommended that the FQPA safety factor for protection of infants and children (as required by FQPA) should be retained at 10x when assessing acute dietary and short-/intermediate-term residential (non-occupational) exposures and reduced to 3x when assessing chronic dietary and long-term residential (non-occupational) exposures resulting from the use of acifluorfen.

A. Acute Toxicity

Table 1 summarizes the acute toxicity data for acifluorfen. Acifluorfen is not acutely toxic *via* the oral [rats], dermal [rabbits], or inhalation [rats] routes of exposure in the studies required for labeling. In guinea pig, acifluorfen is not a skin sensitizer. However, it caused severe eye and moderate skin irritation in the rabbit.

Table 1. Acute Toxicity of Acifluorfen Technical[a]

Guideline No.	Study Type	MRIDs #	Results	Toxicity Category
81-1	Acute Oral (rats)[b]	00071887	LD_{50} =1540 mg/kg	III
	(dog)[b]	00071889	LD_{50} = 186 mg/kg	II
81-2	Acute Dermal (rabbits)	00122725	LD_{50} > 2000 mg/kg	III
81-3	Acute Inhalation	00122726	LC_{50} > 6.9 mg/L	IV
81-4	Primary Eye Irritation	00126597	Severe eye irritant	I
81-5	Primary Skin Irritation	00126597	Moderate dermal irritant	II
81-6	Dermal Sensitization	00122728	Not a skin sensitizer	

a: a 20.2-23.25% (W/V) aqueous dispersion of Acifluorfen technical (TACKLE)
b: a 40% (W/V) aqueous dispersion of Acifluorfen technical (BLAZER)

The above studies satisfy the acute toxicity data requirements (OPPTS 870.1100-870.1300, 870.2400-870.2600; formerly §81-1 through §81-6) for acifluorfen.

B. Subchronic Toxicity

Available studies are adequate to satisfy subchronic testing requirements for acifluorfen. The 21-day dermal toxicity study in rabbits showed dermal irritation at all doses tested and 19/20 animals in high dose group (1000 mg/kg/day) died by day 8. The subchronic feeding study in rats and mice showed decrease in body weight and liver toxicity such as increased liver weight and increased incidence of cellular hypertrophy. There is no subchronic toxicity in dogs on acifluorfen. However, there was an acceptable chronic feeding toxicity study in dogs. Under this circumstance, a subchronic toxicity in dogs is not required.

Dermal toxicity in rabbits [21-day]

In a 21-day dermal toxicity study (MRID No. 00122731; Accession No. 071311), Tackle (21.1 % a.i.) was applied dermally to normal (5/sex/group) and abraded (5/sex/group) skin sites of New Zealand White rabbits at levels of 0, 100, 300 or 1000 mg/kg/day. The test animals were exposed to the chemical for 6 hours/day and 5 days/week. Application of 1000 mg/kg/day resulted in death or moribund sacrifices (19/20) by day 8 of the study. Prior to death, clinical signs, such as respiratory difficulty, excessive salivation, ataxia and tremor were seen in the dying animals. No changes were seen in the body weight, food consumption, hematology, clinical chemistry and urinalysis parameters for any groups that survived to the end of the study. Dermal irritation was observed in all treated animals beginning on day 2 and continuing throughout the study, and the irritation was characterized by hemorrhage, discoloration, crust formation, focal scars, dryness, scales and other changes at the treatment sites. Histopathologically, all treated animals had eschar formation, necrosis, ulceration, epidermal thickening (acanthosis), hyperkeratosis, acute and chronic inflammation, and fibrosis on the areas of the treated skin.

The NOAEL for systemic toxicity is 300 mg/kg/day and LOAEL is 1000 mg/kg/day based on mortality of 19/20 animals (both sexes) by day 8 of the study. The NOAEL for skin irritation is not established and LOAEL is equal to or less than 100 mg/kg/day, (LDT).

This study is classified as Acceptable/Guideline and satisfies the Subdivision F guideline requirement for a 21-dermal toxicity study in rabbits (82-2).

Subchronic toxicity in rats

In a 90-day feeding study (MRID No. 00122730; Accession No. 071308), Tackle (20.4-23.6% a.i.) was administered to Fischer 344 rats (30/sex/dose) in the diet at levels of 0, 20, 80, 320, 1250, 2500 or 5000 ppm (0, 2, 8, 32, 125, 250 or 500 mg/kg/day based on a conversion factor of 1 ppm = 0.1 mg/kg/day) for 3 months. Body weights for 2500 and 5000 ppm males and 5000 ppm females were decreased (92.3-95.5%, 65.3%-71.2% and 83.3-90.6% of the controls, respectively). Food consumption for the 5000 ppm males and females was decreased throughout most of the study period.

At 2500 ppm or above in one or both sexes, there were significant ($p<0.05$) changes in hematology parameters (erythrocyte counts, hematocrit, and hemoglobin concentration), clinical chemistry values (serum electrolytes, calcium, phosphorus, glutamic pyruvic transaminase and alkaline phosphatase activities, BUN, creatinine, serum protein, albumin and globulin concentrations) and urinalysis parameters (nitrate and urobilinogen content). Liver and kidney weights (both absolute and relative) were increased. Histopathological changes were seen in the liver of 2500 ppm or above males and females (including increased cellular hypertrophy, mitotic activity, individual cell death, and proliferation of oval or bile duct cells).

In the 1250 ppm group (in one or both sexes), there were decreases in hematology parameters (erythrocyte counts and hematocrit values), increase in absolute and relative liver weights (20-21% and 22-74%, respectively), increase in absolute and relative kidney weights (11-13% and 10-12%, respectively), and increased incidence of hypertrophy of liver cells when compared to the controls.

No significant treatment-related effects were seen in males or females of 320 ppm or below. The LOAEL for subchronic toxicity is 1250 ppm (125 mg/kg/day) based on decreases in hematology parameters, increases in liver and kidney weights, and increased incidence of hypertrophy of liver cells when compared to the controls. The NOAEL for systemic toxicity is 320 ppm (32 mg/kg/day).

NOTE: In the original DER [Tox. Doc. No. 003409 (same as 003556)], this study was classified as supplementary. The registrant was requested to submit the following: a) Individual animal histopathology on the animals of the 20-2500 ppm groups; b) An explanation of why in the "author's report the least frequent clinical observation was "loss of hair" while the submitted daily record of clinical signs did not mention this event [pages 98-102 of the report].

In the Agency's response to registrant's comment on previous review, this study was classified as supplementary (Tox. Doc. 003963). The explanation of regarding "loss of hair" was satisfactory but the individual animal histopathology on the animals of the 20-2500 ppm groups was not submitted.

This study is classified as Unacceptable/guideline but upgradable. The data in this study was used to set the doses in the rat chronic/carcinogenicity study.

Subchronic toxicity in mice

In a 90-day feeding study (MRID No. 00252826; Accession No. 071308), Tackle (20.4-23.2% a.i.) was administered to B6C3F1 mice (30/sex/dose) in the diet at levels of 0, 20, 80, 320, 1250 or 2500 ppm (0, 3, 12, 48, 187.5 or 375mg/kg/day based on a conversion factor of 1 ppm = 0.15 mg/kg/day) for 3 months. Changes in mean body weights, hematology (total white blood cell numbers, MCV, reticulocyte counts), clinical chemistry (SGPT, alkaline phosphatase, serum glucose), liver weights (absolute and relative), and histopathological changes in the liver (hypertrophy, increased mitotic

activity, individual cell death and focal necrosis) were noted in males and females at the 2500 and 5000 ppm doses at 30 and 90 days. Fatty infiltration of the liver was observed in males and females at the 1250, 2500 and 5000 ppm doses at 30 days and at the 1250 and 2500 doses at 90 days.

The NOAEL for systemic toxicity is 320 ppm (48 mg/kg/day) and the LOAEL is 1250 ppm (187.5 mg/kg/day) based on histopathologic changes (fatty infiltration) of the liver. This study is classified as Unacceptable/guideline but upgradable. The data in this study were used to select the doses in the mouse chronic/carcinogenicity study.

C. Chronic Toxicity [feeding]

Available studies are adequate to satisfy chronic toxicity and carcinogenicity testing requirements for acifluorfen. The chronic feeding toxicity study in rats, mice and dogs demonstrated that acifluorfen induced liver toxicity (acidophilic cells in the liver and increased liver weight) and kidney toxicity (nephritis/pyelonephritis and increased kidney weight). The carcinogenicity data showed that acifluorfen produced an increase in incidence of liver and stomach tumors in mice but not in rats. Acifluorfen was classified as a Group B, probable human carcinogen, and the unit risk [q^*_1] was calculated to be 3.55×10^{-2} $(mg/kg/day)^{-1}$ [The HED Cancer Peer Review Committee memo dated March 17, 1988; HED Doc. No. 007698].

Chronic toxicity in dogs

In a chronic toxicity study (MRID No. 00131162; Accession No's.251297 and 251298), Tackle "2S" (Acifluorfen, sodium salt; purity was unspecified) was administered to Beagle dogs (8/sex/dose) in the diet at levels of 0, 20, 300 or 4500 ppm (0, 0.5, 7.5 or 112.5 mg/kg/day based on a conversion factor of 1 ppm = 0.025 mg/kg/day) for 2 years.

Body weights were lower than respective controls in males (-8 to -20%) and females (-9 to -10%) at 4500 ppm (high-dose) throughout the study (statistical analysis was not performed). In addition, increases in liver and kidney weights (both absolute and relative) were seen in high dose males and females. Additionally, in both sexes at high dose, red blood cell counts, hemoglobin, hematocrit and cholesterol values were significantly lower and leukocyte counts and urinary volume were significantly higher than those of the controls. In males at high dose, serum level of creatinine was lower than that of the controls. Platelet counts, lactate dehydrogenase activity and specific gravity of urine were higher than respective controls. In females at high dose, serum levels of calcium and albumin were lower when compared to the controls. Histologically, there were increased incidence of microscopic changes in the liver (congestion, brown pigment, fatty vacular and inflammation) when compared to the controls.

The LOAEL for systemic toxicity is 4500 ppm (112.5 mg/kg/day) based on decreased body

weight gain, increased absolute and relative liver and kidney weights, changes in hematology, biochemistry, and urinalysis parameters and increased incidence of microscopic changes in the liver. The NOAEL for systemic toxicity is 300 ppm (7.5 mg/kg/day).

This chronic feeding study in dogs is **classified as Unacceptable/guideline and does not satisfies the guideline data requirement for a chronic toxicity study (83-1) in dogs. However, it can be upgraded.** Reasons for this classification are the purity of the test substance was not adequately identified in the report and stability of the test substance in dog chow was not reported. However, **a new study is not required** because when this study and the data of another chronic feeding study in dog (MRID No. 00107484) with Blazer are analyzed together, they provide a reasonable understanding of the chronic toxicity of acifluorfen in dogs. Another chronic feeding study in dog (MRID No. 00107484) with Blazer is an old study (reported in 1978) which was classified as core guideline data, however, the DER for this study was not available. In the old study, there were changes in hematological and biochemical parameters related to the effects in the liver of the treated dogs at high dose (1800 to 5400 ppm) when compared to the controls. Histopathologically, treatment related alterations were observed in the liver, kidney, gall bladder and eyes when compared to the controls. The NOAEL for systemic toxicity was 50 ppm and LOAEL was 300 ppm (mid dose) based on a coagulation effects when compared to the controls (HED Doc. No. 001099; extracted from the review of A. Arce dated May 2, 1979).

Chronic toxicity/carcinogenicity in mice [feeding]

(1)

In a 24-month carcinogenicity study (MRID No. 00082897), Blazer (39.4-40.5% a.i.) was administered to CR CD-1 mice (80/sex/dose) in the diet at levels of 0, 7.5, 45 or 270 ppm for 24 months (0, 1.125, 6.75 or 40.5 mg/kg/day based on a conversion factor of 1 ppm = 0.15 mg/kg/day). The highest dose level was initially administered to mice at a dose of 1.25 ppm on study weeks 1 to 16 before being increased to 270 ppm. Blazer produced a statistically significant ($p<0.05$) increase in the total number of liver tumors (primarily adenomas) in high dose (270 ppm) female mice. No significant increase in liver tumors occurred in male mice.

In high dose males, there was a dose-related increase in absolute and relative liver weights and relative kidney weights. In addition, there was dose related elevation of alkaline phosphatase and serum glutamic pyruvic transaminase activities in mid and high dose male mice. However, due to the variability of the standard deviation for these parameters, statistical analysis was not performed.

The NOAEL for systemic toxicity is 45 ppm (6.75 mg/kg/day) and the LOAEL is 270 ppm (40.5 mg/kg/day) based on increased absolute and relative liver weights, increased relative kidney weights, and increased levels of alkaline phosphatase and glutamic pyruvic transaminase activities.

This study by itself does not satisfy the guideline requirement for a carcinogenicity study (83-2b) in mice. The deficiencies of the study report are as follows: resolution of histopathology questions; explanation of why the 270 ppm diet was analyzed only once; provide stability of the compound in feed data; explanation of Table 4 (Food Consumption). However, the requirement for carcinogenicity study for mice is satisfied when considered together with the other mouse carcinogenicity study (MRID No. 00122732; Accession No. 071312, 071313, 071314, 250463, and 250464). When the data of these two studies are analyzed together, they provide a reasonable understanding of the carcinogenicity of acifluorfen on mice.

(2)

In a 18-month carcinogenicity study (MRID No. 00122732; Accession No's.071312, 071313, 071314, 250463, and 250464), Tackle (20.4-23.2% a.i.) was administered to B6C3F1 mice (60/sex/dose) in the diet at levels of 0, 625, 1250 or 2500 ppm (0, 119, 259 or 655 mg/kg/day for males and 0, 143, 313 or 711 mg/kg/day for females) for 18 months.

An increase in mortality was seen in high dose males (control, 1/60; high-dose, 10/60). Body weights in all treated mice were reduced when compared to controls. Beginning at week 2 for mid- and high-dose males and week 6 for low-dose males, body weights were significantly reduced ($p<0.01$) relative to controls. The body weight decrease for low-, mid-, and high-dose males was 8%, 10% and 23% of the controls, respectively at week 13 and 10%, 13% and 25% of the controls, respectively at week 79. Similar results were obtained with females, except that reduced body weights of low- and mid-dose females were not significant until week 13. The body weight decrease for low-, mid-, and high-dose females was 6%, 5% and 14% of the controls, respectively at week 13 and 11%, 22% and 34% of the controls, respectively at week 79. Mean food consumption in high-dose males was higher relative to the controls most of the study period.

Mean corpuscular volume (MCV) and segmented neutrophil counts were decreased and lymphocyte and RBC counts were increased compared to controls in all treated males at final sacrifice. Segmented neutrophil counts was decreased and lymphocyte counts were increased in all treated females (at interim sacrifice) and in mid- and high-dose females (at final sacrifice) when compared to controls.

Mean absolute and relative liver weights of treated males and females were greater than the controls at interim and final sacrifice. Gross pathology showed an apparent dose-related increase in incidence of liver masses in treated males when compared to the controls at final sacrifice. In high dose females, an increase in incidence (37%) of liver masses was observed. The incidence of white foci (1 mm) on the nonglandular portion of the stomach were seen in high-dose males and mid- and high-dose females. In addition, one high-dose male and one high-dose female each had an ulcer of the stomach.

Acifluorfen was associated with statistically significant positive trends for liver tumors (adenomas, carcinomas, and adenomas/carcinomas combined) and stomach tumors (papillomas) in both sexes.

The liver tumors were significantly increased above the controls at the lowest dose level tested (625 ppm) in male mice and at the highest dose level tested (2500 ppm) in both sexes. In addition, the stomach tumors were significantly increased above the controls at the highest dose level tested (2500 ppm) in both sexes. The highest dose tested was considered adequate in evaluation of carcinogenic potential of the test chemical.

The NOAEL for systemic toxicity is not established. The LOAEL for systemic toxicity is equal or lower than 625 ppm (119 and 143 mg/kg/day for males and females, respectively) based on reduced body weight, increased absolute and relative liver weights, and changes in hematologic parameters (decreased MCV counts, decreased segmented neutrophil counts, increased RBC counts, and increased lymphocyte counts).

This study is classified as Acceptable/Guideline and satisfies the Subdivision F guideline requirement for a carcinogenicity study (83-2b) in mice.

Chronic toxicity/carcinogenicity in rats [feeding]

In a two-year feeding/carcinogenicity study (MRID No. 00128253; Accession No's. 071315 through 071317 and 250289 through 250792), Tackle (19.1-25.6% a.i.) was administered to Fischer 344 rats (73/sex/dose) in the diet at levels of 0, 25, 150, 500, 2500 or 5000 ppm for 2 years (0, 1.25, 7.50, 25.0, 125 or 250 mg/kg/day based on a conversion factor of 1 ppm=0.05 mg/kg/day).

All males and 61/65 females in 5000 ppm group died before termination of the study. Mean body weight was significantly decreased in males at the 2500 (7-9%) and 5000 ppm (18-35%) throughout the study relative to the controls. In females, the body weight was also decreased in 2500 (6-16%) and 5000 ppm (11-28%) groups relative to the controls.

Red cell counts, hematocrit and hemoglobin values were significantly lower in the 5000 ppm males when compared to the controls.

In the 5000 ppm males and females, blood glucose, triglyceride, and serum globulin and total protein were significantly lower than those of the control animals; BUN, creatinine and alkaline phosphatase were significantly higher than those of the control animals. In the 2500 ppm males, blood glucose, triglyceride and globulin were significantly lower and BUN was significantly higher when compared to the control animals. In the 2500 ppm females, triglyceride and globulin were significantly lower and alkaline phosphatase was significantly higher when compared to the control animals.

Gross necropsy showed that there was an increased incidence of kidney and liver discoloration, stomach ulcers and reduced testes size for the 5000 ppm males when compared to the controls. In females, an increased incidence of kidney lesions (distended pelvis/calculi) was observed in the 2500

and 5000 ppm animals and stomach ulceration was observed in the 5000 ppm animals when compared to the controls.

In 2500 ppm males, there were increases in absolute and relative liver weights, decreases in spleen weights, and increases in relative kidney weights. In 5000 ppm males, there were increases in liver weights and decreases in spleen weights. In females at 2500 and 5000 ppm, there were increases in relative liver weights and decreases in heart weights.

Histologically, there were increased incidences of acidophilic cells in the liver of the 5000 ppm group in both sexes and in the 2500 ppm females. In addition, there were increased incidences of nephritis/pyelonephritis in 2500 and 5000 ppm group females. An increase in the incidence of stomach ulcer was seen in 5000 ppm males and females. Testicular atrophy was also seen in 5000 ppm males.

Under the condition of the study, no treatment-related increase in tumor incidence was found in the acifluorfen treated rats.

The NOAEL for systemic toxicity is 500 ppm (25 mg/kg/day); the LOAEL is 2500 ppm (125 mg/kg/day) based on reduced body weight, increased absolute and relative liver weights and increased kidney weights, increased incidence of nephritis/pyelonephritis, increased incidence of acidophilic cells in the liver, and related changes in clinical chemistry parameters

The highest dose (5000 ppm) tested was considered excessively toxic in evaluation of carcinogenic potential of the test chemical based on findings of reduced body weights, increased mortality, increased liver weights and liver enzyme changes (alkaline phosphatase), renal changes (nephritis and pyelonephritis), stomach ulcers and decreased testes size.

This study is classified as Acceptable/guideline and satisfies the guideline data requirement for a combined chronic/carcinogenicity study (83-5) in rats.

Another rat chronic feeding study (MRID No. 00087478) with Blazer was also conducted by Rohm and Haas. The DER (HED Doc. No. 001099 and 001251) for this study indicated no increase in any tumor incidence in treated animals relative to the controls. However, the experimental design of the study used multiple shifts in dose levels at different durations of the study. It is difficult to establish at what dose levels or times that changes in biological parameters occurred. However, when the data of these two studies are analyzed together, they provide a reasonable understanding of the chronic toxicity of acifluorfen in rats.

D. Developmental/Reproductive Toxicity

Available developmental toxicity studies and reproduction study are adequate to satisfy guideline requirements. Acifluorfen produced developmental toxicity (decreased fetal body weight and the

increase in anatomical variations) in rats but not in rabbits and it did not affect reproductive parameters in rats.

Developmental toxicity in rats

In a developmental toxicity study (MRID No.00122743; Accession No. 071319), Tackle "2S" (22.4% a.i.) was administered to Crl:COBS CD (SD) BR rats (25/sex/dose) by intubation at doses of 0, 20, 90, or 180 mg/kg/day from gestation days 6 through 19. Three animals from the high-dose group died on test.

Clinical signs, such as excessive salivation, urine-stained fur of the abdomen, rales, decreased motor activity and chromodacryorrhea were observed in the high-dose animals. Clinical signs, excessive salivation and piloerection, were also observed in the mid-dose animals.

The mean body weights of the high-dose animals was significantly ($p<0.01$) decreased from gestation day 13 to sacrifice. The change in body weights of the high-dose animals over the treatment period (days 6-19) (-35%) and over the entire gestational period (days 0-20) (-26%) were lower than the control values. **The NOAEL for maternal toxicity is 20 mg/kg/day. The LOAEL for maternal toxicity is 90 mg/kg/day based on increase in clinical signs.**

A significant increase ($p < 0.0001$) in resorptions in the high-dose group and significant reduction ($p< 0.01$) in mean fetal weights for both the mid- and high-dose groups were observed. A significant increase ($p< 0.05$ or 0.01) in anatomical variations was seen in the mid- and high-dose groups. The variations included slightly dilated lateral ventricles of the brain, hemorrhage in the eyeball, slight dilation of the renal pelvis, hemorrhage in either the peritoneal cavity or subcutaneous spaces, and minor changes in ossification (such as incomplete ossification of supra-occipital sternebra or thoracic centra). **The NOAEL for developmental toxicity is 20 mg/kg/day; the LOAEL for developmental toxicity is 90 mg/kg/day based on the decreased fetal body weight and the increase in anatomical variations.**

This study is classified as ACCEPTABLE/GUIDELINE and satisfies the guideline data requirement for a developmental study (83-3a) in rats.

Developmental toxicity in rabbits

(1)

In a developmental toxicity study (MRID No. 00122744; Accession No. 071319), Tackle "2S" (22.4% a.i.) was administered to New Zealand White rabbits (16/sex/dose) by intubation at doses of 0, 3, 12 or 36 mg/kg/day from gestation days 6 through 29.

No statistically significant differences were noted in maternal body weights, body weight gains, numbers of corpora lutea, implantations/litter, resorptions, pregnancy rate or implantation rate among all groups. Based on these data, **the NOAEL for maternal toxicity for acifluorfen is equal to or greater than 36 mg/kg/day (highest dose tested) and the LOAEL is not established.**

No evidence of treatment-related effects on mean fetal weights, anatomical variations, malformation, or alterations in sex ratios of surviving fetuses were seen.

Under the conditions of this study, **the NOAEL for developmental toxicity is equal to or greater than 36 mg/kg/day (highest dose tested) and the LOAEL is not established.**

The developmental toxicity study in the rabbit is classified as **Acceptable/guideline** and satisfies the guideline requirement **(§83-3b)** for a developmental toxicity study in the rabbit **only in conjunction with the 1976 study (MRID No. 00107485) (see below)**.

(2)

In another developmental toxicity study (MRID 00107485), sodium acifluorfen (39.8% a.i.) in an aqueous solution was administered by gavage at 0, 20, 60 or 180 mg/kg/day to pregnant rabbits (15/dose) during gestation days (GDs) 7 through 19. At GD 29, surviving does were sacrificed and necropsied.

There were no differences of toxicological concern in pregnancy rate and numbers of implantations.

At 180 mg/kg/day, does exhibited increased mortality (11 treated vs 1 control; 8 animals died during treatment [GDs 7 through 19] and 3 animals died after treatment). Clinical signs observed in the high dose group included anorexia (15/15), depression (12/15), cyanosis (6/15), dyspnea (4/15), and thinness (9/15) during treatment (GDs 7 through 19). During the posttreatment period (GDs 20-29), high-dose does showed increased depression (7/7 treated vs 1/15 controls), dyspnea (4/7 treated vs 1/15 controls), cyanosis (7/7 treated vs 0/15 controls), and thinness (7/7 treated vs 0/15 controls). Body weight was not significantly different between groups, although it was decreased in the high-dose group compared to controls from GD 10 through GD 29 (911-30%). Body weight gains were significantly decreased during dosing (GDs 7 through 19, 952 x, $p<0.05$) and overall (95.6x, $p<0.05$) in the high-dose group compared to controls. There were no live fetuses in the 3 remaining high-dose does. As a result, the incidences of resorption (8300%) and fetal viability (9100%) were statistically different from the controls ($p<0.05$).

Three of the 60 mg/kg/day does died. Two died due to technician errors and the cause of death of the third animal was not reported. Gross pathology of the 3 animals was not remarkable. Anorexia (9/15), depression (5/15) and dyspnea (1/15) were also observed in this group during the treatment period.

There were no treatment related differences observed in the low-dose group.

The NOAEL for maternal toxicity is 20 mg/kg/day and the LOAEL is 60 mg/kg/day based on clinical signs.

All conceptuses in surviving high-dose group were resorbed. Total resorption precluded examination of fetuses in the high-dose group. There were no treatment-related effects in developmental parameters observed in the mid- and low-dose groups.

The NOAEL for developmental toxicity is 60 mg/kg/day and the LOAEL is 180 mg/kg/day based on fetal resorptions.

The developmental toxicity study in the rabbit is classified as **Unacceptable/guideline, but upgradable** and does not satisfy the guideline requirement **(§83-3b)** for a developmental toxicity study in the rabbit. It was not stated how often the dose formulations were prepared. No concentration or stability data were submitted, therefore there is no way to verify the actual concentration of the test material given to the does.

However, the requirement for developmental toxicity in rabbit is satisfied when considered together with the other rabbit developmental toxicity study (MRID No. 00122744; HED Doc. No. 003556; 1980 study). When the data of these two studies are analyzed together, they provide a reasonable understanding of the developmental toxicity of acifluorfen in rabbit. The 1980 study showed no maternal or embryo/fetal toxicity at 36 mg/kg/day (HDT) and the 1976 study showed maternal and embryo/fetal toxicity and NOAEL/LOAEL for both.

Reproductive toxicity in rats

In a 2-generation reproduction study (MRID No.00155548), Tackle "2S" (21.1-21.6% a.i.) was administered to Crl:COBS CD (SD) BR Rats (35/sex/dose) in the diet at levels of 0, 25, 500 or 2500 ppm (0, 2.5, 50 or 250 mg/kg/day based on a conversion factor of 1 ppm = 0.1 mg/kg/day).

During the premating period, body weights and/or body weight gains of P1 and F1 high-dose males and females were significantly decreased ($p<0.05$); the body weight decrease in high dose F1 females persisted throughout gestation and lactation (10-25% at various times of determination). In contrast, the food consumption in high dose animals was greater than that of the controls at majority of the measuring intervals. The food consumption in mid- and low-dose groups was comparable to that of the controls.

The test chemical did not significantly affect any of the reproductive parameters.

Microscopically, in both generations P1 and F1 females, administration of Tackle at mid- and high-doses resulted in increased incidences of kidney lesions, characterized predominantly by dilatation of tubules in the outer medulla. In high-dose second generation P1 males, there was a significant increase in the incidence of pelvic dilatation (hydronephrosis) when compared to the controls.

In both generations of offspring, the body weight was significantly lower for high-dose litters (6-19% decrease for F1 and 11-26% decrease for F2) at birth and on lactation days 7, 14, and 21 when compared to the controls. Other offspring effects observed were as follows: Viability indices (percentage of liveborn pups that survived to day 4 postpartum) were lower in F1 generation only when compared to the controls. In F2 generation, the incidence of pups dying between lactation days 1 and 4 was significantly increased (3-3.4%) for the mid- and high-dose groups when compared to the controls. In addition, in F2 generation, the incidence of grossly observed kidney lesions, primarily dilatation of the pelvis, was significantly increased at the high-dose level when compared to the controls.
The NOAEL for parental toxicity is 25 ppm (2.5 mg/kg/day) and the LOAEL is 500 ppm (50 mg/kg/day) based on kidney lesions, characterized predominantly by dilatation of tubules in the outer medulla, in females of both generations.

The NOAEL for reproductive toxicity is equal to or greater than 2500 ppm (250 mg/kg/day) the highest dose tested and the LOAEL is not established.

The NOAEL for offspring toxicity is 25 ppm (2.5 mg/kg/day) and the LOAEL is 500 ppm (50 mg/kg/day) based on decreased viability and increased incidence of kidney lesions, characterized predominantly by dilatation of pelvis in F2 generation.

This study is classified as Acceptable/Guideline and satisfies the guideline data requirement for a multi-generation reproduction study (83-4) in rats.

E. Mutagenicity

The acceptable genetic toxicology studies indicate that sodium acifluorfen was weakly mutagenic in *Salmonella typhimurium* TA100 at high S9-activated concentrations and weakly recombinogenic in *Saccharomyces cerevisiae* at high nonactivated concentrations but was negative for gene mutations in Chinese hamster ovary (CHO) cells. The test material was also negative for clastogenic effects *in vivo* and did not induce unscheduled DNA synthesis in primary rat hepatocytes. An *in vivo* dominant lethal assay in rats yielded negative results. The acceptable studies satisfy the pre-1991 mutagenicity guideline requirements. Because the biological relevance of the positive bacterial assay data is not clear, it is recommended that sodium acifluorfen be test in the pre-incubation modification to the

Salmonella typhimurium mammalian microsome gene mutation assay. Summaries of the submitted acceptable mutagenicity studies are presented below:

GENE MUTATIONS

1) *Salmonella typhimurium* mammalian microsome gene mutation assays: Sodium acifluorfen (42.8% active ingredient, a.i) was weakly positive with significant and reproducible, but less than 2-fold increases in histidine revertants of strain TA100 at high concentrations (4000 and 5000 Fg/plate) with S9 activation. The study is classified as Acceptable/guideline and satisfies the requirements for FIFRA Test Guideline 84-2 for a bacterial gene mutation assay. However, because the biological relevance of the finding is not clear, it was recommended that the assay be repeated using the pre-incubation modification to the standard plate incorporation test (MRID No. 41480101).

2) *In vitro* mammalian cell forward gene mutation assay in Chinese hamster ovary (CHO) cells: Sodium acifluorfen (42.8% a.i.) was negative for the induction of gene mutations in independently conducted trials. Cytotoxicity was seen at 650 Fg/mL without S9 activation and at 450 Fg/mL in the presence of S9 activation. The study is classified as Acceptable/guideline and satisfies the requirements for FIFRA Test Guideline 84-2 for a mammalian cell gene mutation assay (MRID No. 41480103).

3) *Drosophila melanogaster* mutagenicity assays: Sodium acifluorfen (1.5% a.i.) was negative for the induction of somatic reversion of the white-ivory mutation in male flies exposed to 1.5% (.15 mg/mL) of the test material for 2 hours, negative for chromosome aberrations (the bithorax test) and sex-linked recessive lethal mutations in male flies exposed to 1.5% of the test material for 24 hours but caused a significant increase in Y chromosome loss and dominant lethal mutations at 1.5% of the test material with a 24-hour exposure. The study is classified as Acceptable/guideline and satisfies the requirements for FIFRA Test Guideline 84-2 for a sex-linked recessive lethal test in *Drosophila melanogaster* (MRID No. 00122737).

CHROMOSOME ABERRATIONS

4) *In vivo* rat cytogenetic assay: Sodium acifluorfen (23.6% a.i) did not induce structural chromosome aberrations in male Sprague Dawley rats administered 0.37, 1.11 or 1.37 g/kg/day for 5 consecutive days by oral gavage. Analysis of blood samples indicated that the test material was absorbed with blood levels of 64.4, 87.8 or 287 Fg/mL, respectively, in treated rats. Lethality was seen in the high-dose group; other evidence of systemic toxicity in high- and mid-dose groups included hypoactivity, labored breathing and increased or persistent urination. Females were not used in this assay, however, the available toxicological data did not indicate that females were more sensitive than males. The study is classified as Acceptable/guideline and satisfies the requirements for FIFRA Test Guideline 84-2 for an *in vivo* cytogenetic assay (MRID No. 00122741).

5) Dominant lethal assay in rats: The test was negative in the germinal cells of male Sprague Dawley rats (sampled over a 7-week period) receiving oral gavage doses of 80, 360 or 800 mg/kg sodium acifluorfen (23.6% a.i.) for 5 consecutive days. With the exception of one death at the HDT, no systemic toxicity was seen. The study is classified as Acceptable/guideline and satisfies the requirements for FIFRA Test Guideline 84-2 for a dominant lethal assay (MRID No.00122738).

OTHER MUTAGENIC MECHANISMS

6) *In vitro* unscheduled DNA synthesis (UDS) assay in primary rat hepatocytes: The test is negative with Sodium acifluorfen (24% a.i.) up to a cytotoxic level (50 Fg/mL). The study is classified as Acceptable/guideline and satisfies the requirements for FIFRA Test Guideline 84-2 for a UDS assay (MRID No. 00122742).

7) *Saccharomyces cerevisiae* D5 mitotic recombination assay: The test was positive; Sodium acifluorfen (29.7% a.i.) induced a weak but dose-related increase in the recombination frequency in the absence of S9 activation at doses ranging from 0.75-2.25 mg/plate. In the presence of S9 activation, the response was not dose-related. The study is classified as Acceptable/guideline and satisfies the requirements for FIFRA Test Guideline 84-2 for a mitotic recombination assay (MRID No. 00148272).

F. Metabolism

Available metabolism data are adequate to satisfy the guideline requirements. With oral administration, acifluorfen was rapidly absorbed and eliminated mainly in the urine (46-58% of the dose) and feces (21-41% of the dose). The major component present in urine and feces was unchanged acifluorfen and amine metabolite, respectively. No tissue accumulation was observed.

(1)

In a rat metabolism study, Fischer 344 rats (5 animals/sex/group) received a single gavage dose of ^{14}C- or -^{13}C-ring-labeled acifluorfen at 16-17 or 116 mg/kg or daily oral dose of non-radioactive acifluorfen-sodium 10-12 mg/kg for 14 days followed by a single gavage dose of ^{14}C- or ^{13}C-ring-labeled acifluorfen at 10-12 mg/kg and an intravenous dosage at 11-15 mg/kg. The single low, single high, and multiple low oral dose studies indicate that acifluorfen is rapidly and almost completely absorbed into the systemic circulation and excreted in both the male and female rats within 4 days after dosing. Over a 4-day period, the radioactivity recovered in the urine and feces in male rats was 46-58% and 21-41% of the dose, respectively. In contrast, the radioactivity recovered in the urine and the feces in female animals was 60-82% and 5-23% of the dose, respectively. There was no indication of bioaccumulation in any tissue or organ after administration of each of the four dosage regimens. The major radioactive component present in blood (95-98%), urine (95%) and bile (93%) was unchanged acifluorfen. The major component in feces was the amine metabolite which accounted for 60-80% of

the radioactivity. This study is classified as Acceptable/guideline and satisfied the guideline data requirement for a metabolism study (85-1) in rats.

(2)

In a non-guideline mouse and rat metabolism studies, non-radioactive acifluorfen was given in the diet at levels of 1.5 mg/kg/day (for CD-1 mice only) and 54 mg/kg/day (for both CD-1 mice and Sprague-Dawley rats) for 28 days. On days 14 and 28 of the study, animals were given a single gavage dose of ^{14}C-acifluorfen at 1.5 mg/kg/day (2 female mice) and 54 mg/kg/day (2 male and female mice and 2 female rats). The dosages chosen for repeated dosing of non-radioactive acifluorfen were equivalent to the low-dose and high-dose in the mouse bioassay (1.5 and 54 mg/kg/day; 7.5 and 270 ppm) and high-dose in the rat bioassay (54 mg/kg/day; 1080 ppm). Urine and feces were collected twice daily for two days and daily thereafter for 2 days. Practically the entire pulse dose of acifluorfen was excreted in the urine and feces within two to three days. Metabolites consisted mainly of free acid (RH-5781; 33-91% of the total dose), reduced amino form of RH-5781 (RH-4514; 11-38%), or origin material (6-35%) consisted of acid hydrolyzable conjugates of RH-5781 and RH-4514. This study is classified as Acceptable/non-guideline. It is acceptable for the purposes for which it was intended as a special study.

G. Neurotoxicity

There are no neurotoxicity studies available for acifluorfen. Similarly, no neurotoxicity studies are available for structurally related compounds (oxyfluorfen, nitrofen, fomesafen, and lactofen). However, there was indication of neurotoxicity in rat fetuses characterized by increased incidence of dilated lateral ventricles of the fetal brain in a developmental toxicity study in rats (MRID No. 00122743). Based on this finding, the HIARC recommended a developmental neurotoxicity study in rats be conducted.

II. Toxicity End-Point Selection

On January 19 and February 11, 1999, the Hazard Identification Assessment Review Committee [HIARC] evaluated the entire toxicological database on acifluorfen and selected the relevant toxicity endpoints, taking into consideration the use patterns and exposure information on this chemical. The selected toxicological endpoints and the doses for risk assessment are summarized in Table 2 and additional relevant details for each endpoint are found in HIARC Report HED Document No. 013308; T:\HED\SARC\HAZID\114402\114402HA.001).

III. FQPA Considerations/Uncertainty Factor

After thorough evaluation of the toxicology database relating to young and developing animals, the HIARC recommended that the FQPA 10X Safety Factor be retained due to the increased sensitivity of offspring observed in the developmental toxicity study in rats. On September 13, 1999, the FQPA Safety Factor Committee evaluated the HIARC recommendation and the available exposure data and concluded that the FQPA safety factor for protection of infants and children (as required by FQPA) should be retained at 10x when assessing acute dietary and short-/intermediate-term residential (non-occupational) exposures for the **Females 13-50** and the **Infants and Children Subgroups**. However, the factor should be reduced to 3x for assessing chronic dietary and long-term residential (non-occupational) exposures for the **Females 13-50** and the **Infants and Children Subgroups**. Additional relevant details are found in the FQPA Safety Factor Committee Report, dated September 13, 1999 [HED Document No. 013764; T:\HED\SARC\FQPA\114402SF.001]

IV. DATA GAPS

The HIARC considered the lack of a developmental neurotoxicity study in rats as the data gap because of the concern of neurotoxic effects in the developmental toxicity study in rats. Evidence of treatment-related anomalies in the development of the fetal nervous system were observed in the prenatal developmental toxicity study in rats at maternally toxic oral dose of 90 mg/kg/day.
Table 2. Doses and Toxicological Endpoints Selected for Various Exposure Scenarios

EXPOSURE SCENARIO	DOSE (mg/kg/day)	ENDPOINT	STUDY
Acute Dietary (Female 13+)	NOEL=20 UF=100	Decreased fetal weight and increased incidences of dilated lateral ventricles of the brain	Developmental–rat
		Acute RfD = 0.2 mg/kg/day	
Acute Dietary (General population)	none	no endpoint established	
	none	none	
Chronic Dietary non-carcinogenic effects	NOEL=1.25 UF=100	based on kidney lesions, characterized predominantly by dilatation of tubules in the outer medulla, in females of both generations	2-generation reproduction–rat
		Chronic =0.013 mg/kg/day	
Carcinogenic effects	$Q^*_1 = 3.55 \times 10^{-2}$ $(mg/kg/day)^{-1}$	liver tumors (adenomas, carcinomas, and adenomas/carcinomas combined) and stomach tumors (papillomas) in both sexes of rats	
Short-Term [a] (Dermal)	NOEL=20 UF=100	Decreased fetal weight and increased incidences of dilated lateral ventricles of the brain	Developmental–rat
Intermediate-Term (Dermal)	NOEL=20 UF=100	Decreased fetal weight and increased incidences of dilated lateral ventricles of the brain	Developmental–rat
Long-Term (Dermal)	None	Based on the registered use, long term dermal exposure is not expected.	
Chronic Dermal non-carcinogenic effects	N/A	N/A	N/A
Inhalation (short & intermediate)[b]	NOEL=20 UF=100	Decreased fetal weight and increased incidences of dilated lateral ventricles of the brain	Developmental–rat
Inhalation (long)	None	Based on the registered use, long term dermal exposure is not expected.	

a = Since an oral NOEL was selected, a dermal absorption factor of 20% should be used in route-to-route extrapolation.
b = Since an oral NOEL was selected, an inhalation absorption factor of 100% (default value) should be used in route-to-route extrapolation.

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